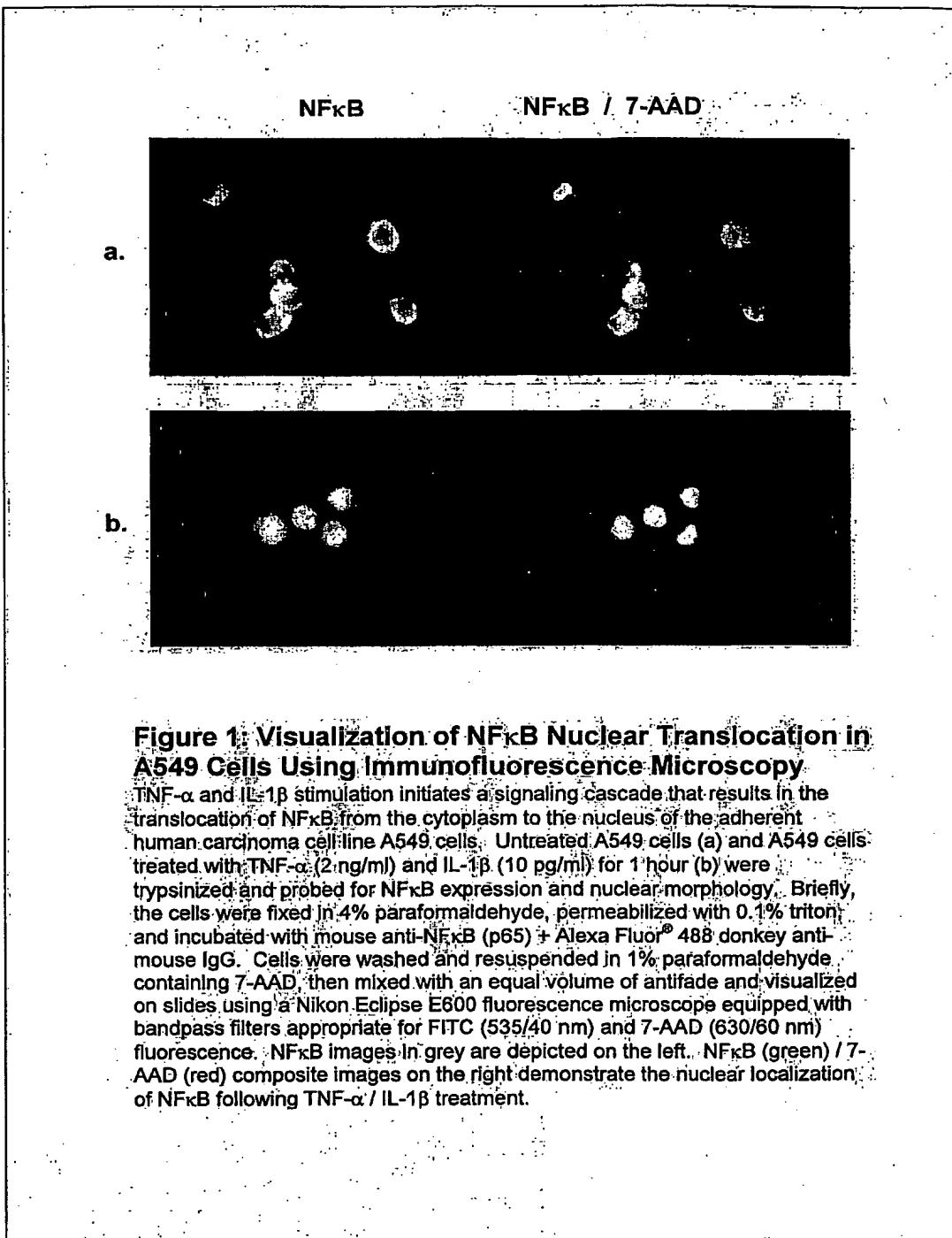


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**Fig. 1**

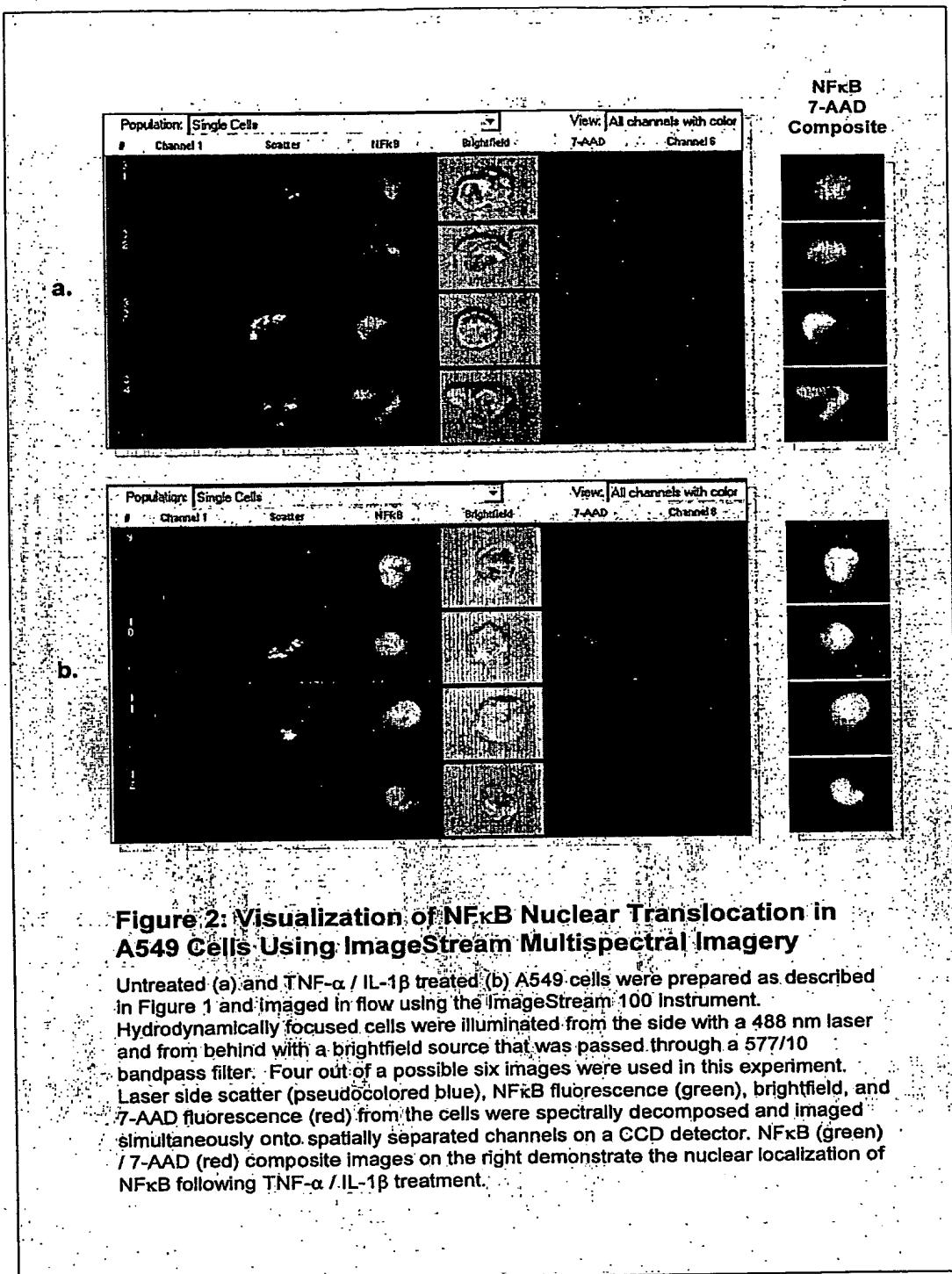


Figure 2: Visualization of NFκB Nuclear Translocation in A549 Cells Using ImageStream Multispectral Imagery

Untreated (a) and TNF- α / IL-1 β treated (b) A549 cells were prepared as described in Figure 1 and imaged in flow using the ImageStream 100 instrument. Hydrodynamically focused cells were illuminated from the side with a 488 nm laser and from behind with a brightfield source that was passed through a 577/10 bandpass filter. Four out of a possible six images were used in this experiment. Laser side scatter (pseudocolored blue), NFκB fluorescence (green), brightfield, and 7-AAD fluorescence (red) from the cells were spectrally decomposed and imaged simultaneously onto spatially separated channels on a CCD detector. NFκB (green) / 7-AAD (red) composite images on the right demonstrate the nuclear localization of NFκB following TNF- α / IL-1 β treatment.

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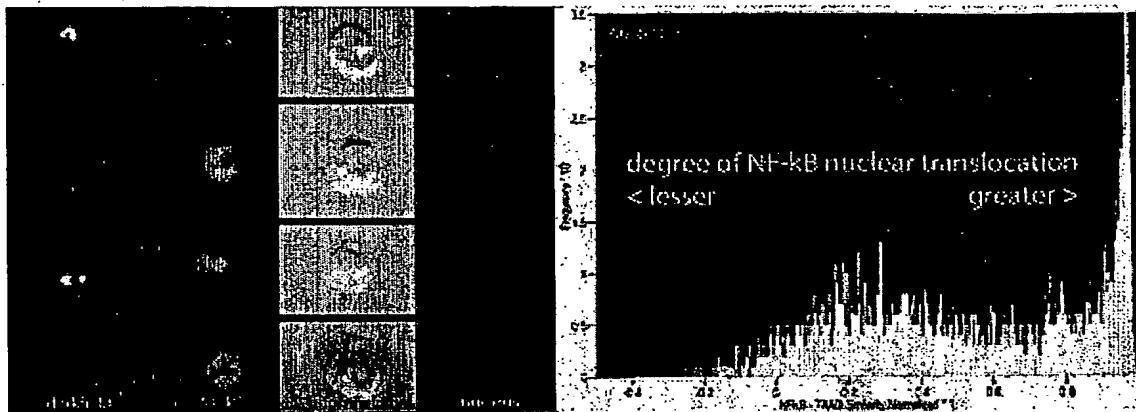
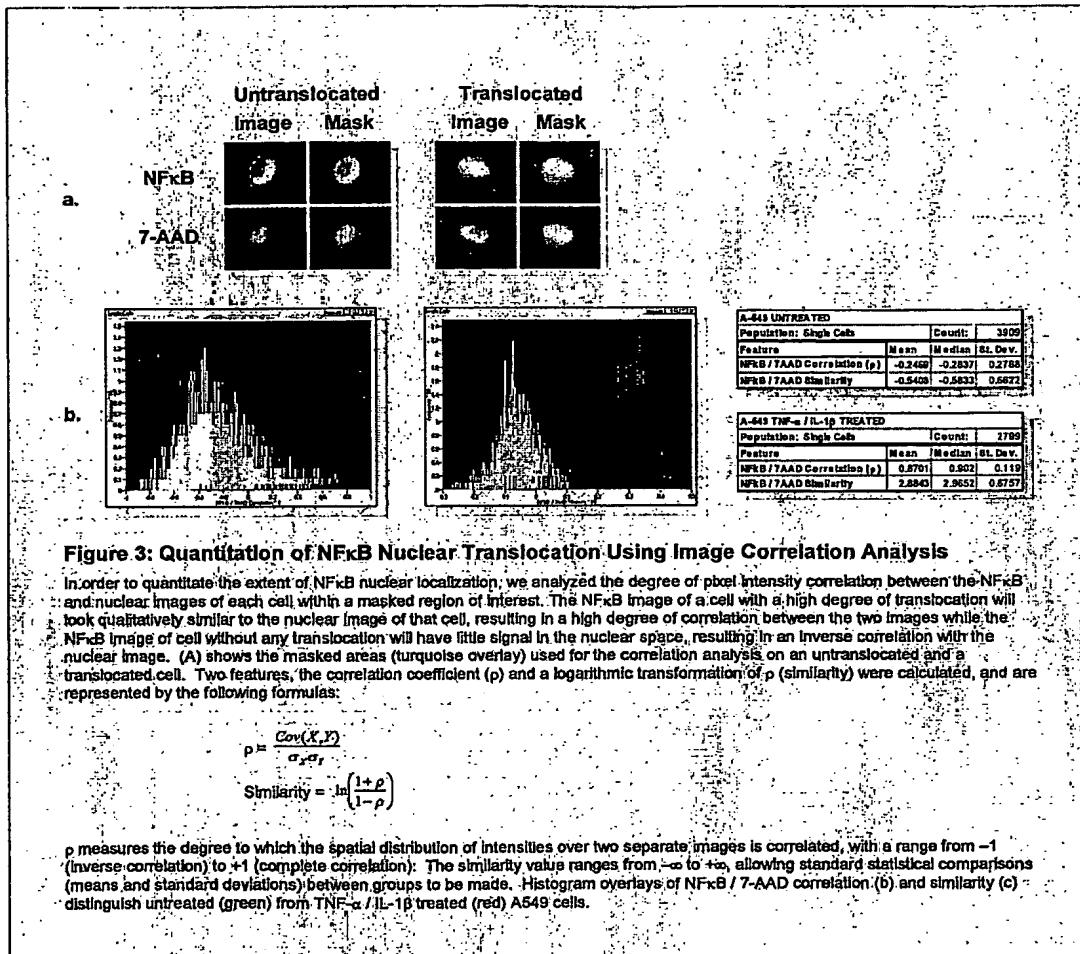


Figure 2C. NF-κB Nuclear Translocation in Immune Cells

The data (above left) show cells imaged simultaneously in darkfield, green fluorescence, brightfield, and red fluorescence. The sample consisted of a monocytic cell line stained with an antibody against the NF-κB transcription factor (green) as well as a nuclear stain (red). Cells treated with lipo-polysaccharide (image rows 2-4) exhibit translocation of NF-κB from the cytoplasm to the nucleus while untreated cells lack NF-κB in the nuclear compartment (top row). A statistical analysis of imagery from 6616 cells quantitatively characterizes the degree of NF-κB nuclear translocation in the sample. Amnis' ImageStream platform is the only cell analysis technology that can perform this valuable assay on immune cells in suspension.

Fig. 2C

**Fig. 3**

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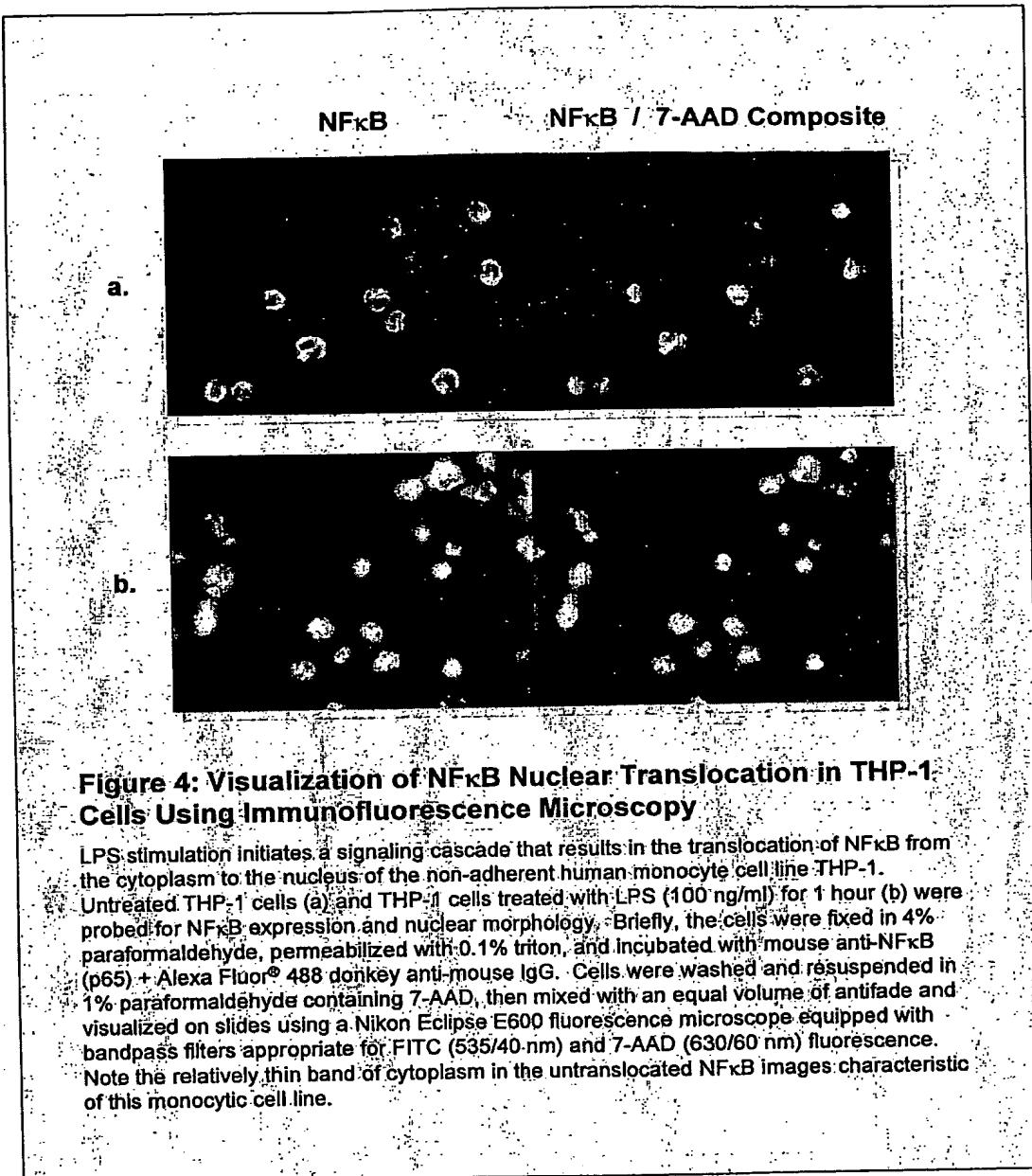


Fig. 4

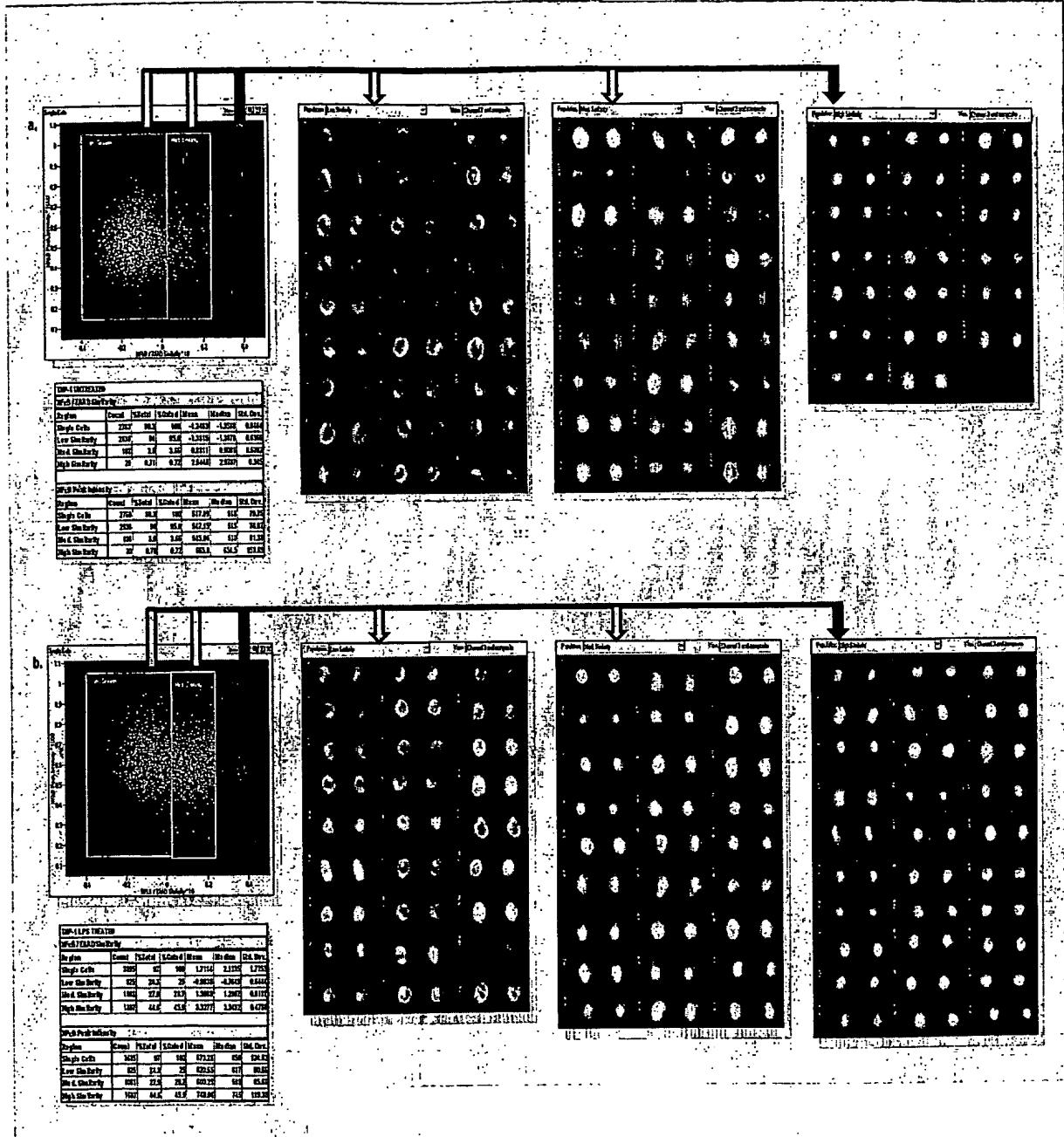


Fig. 5

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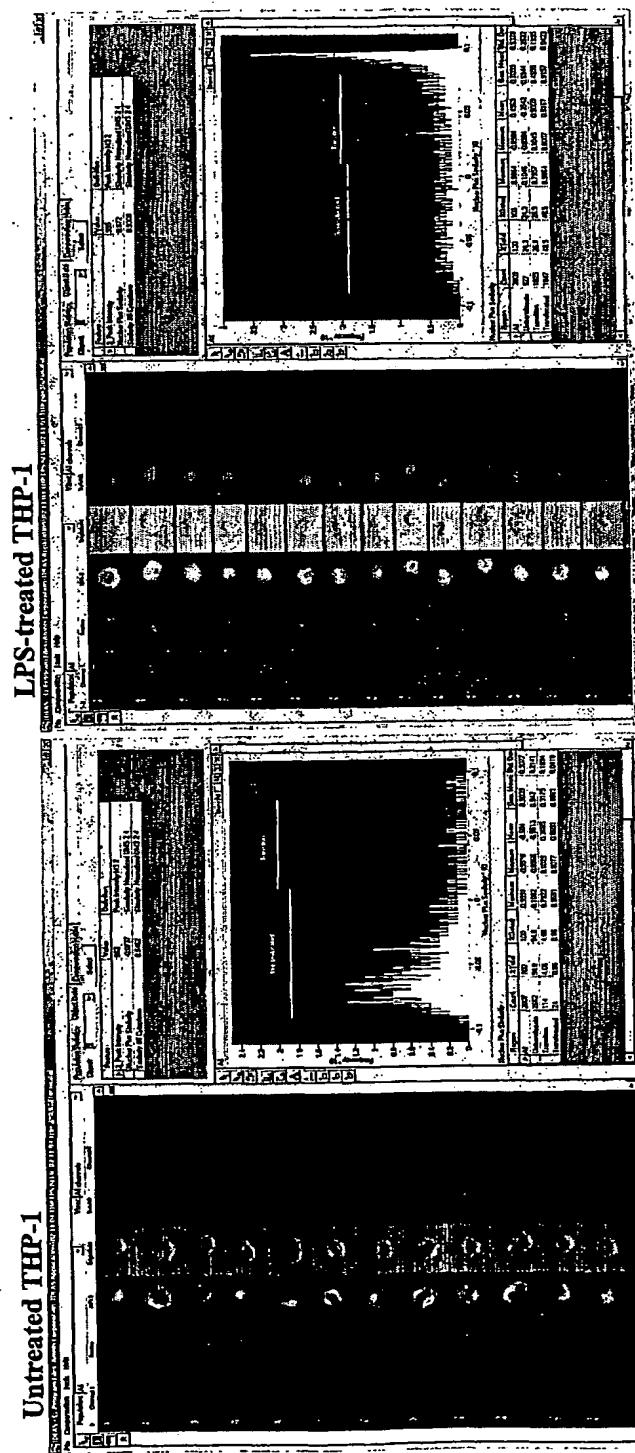


Fig. 6

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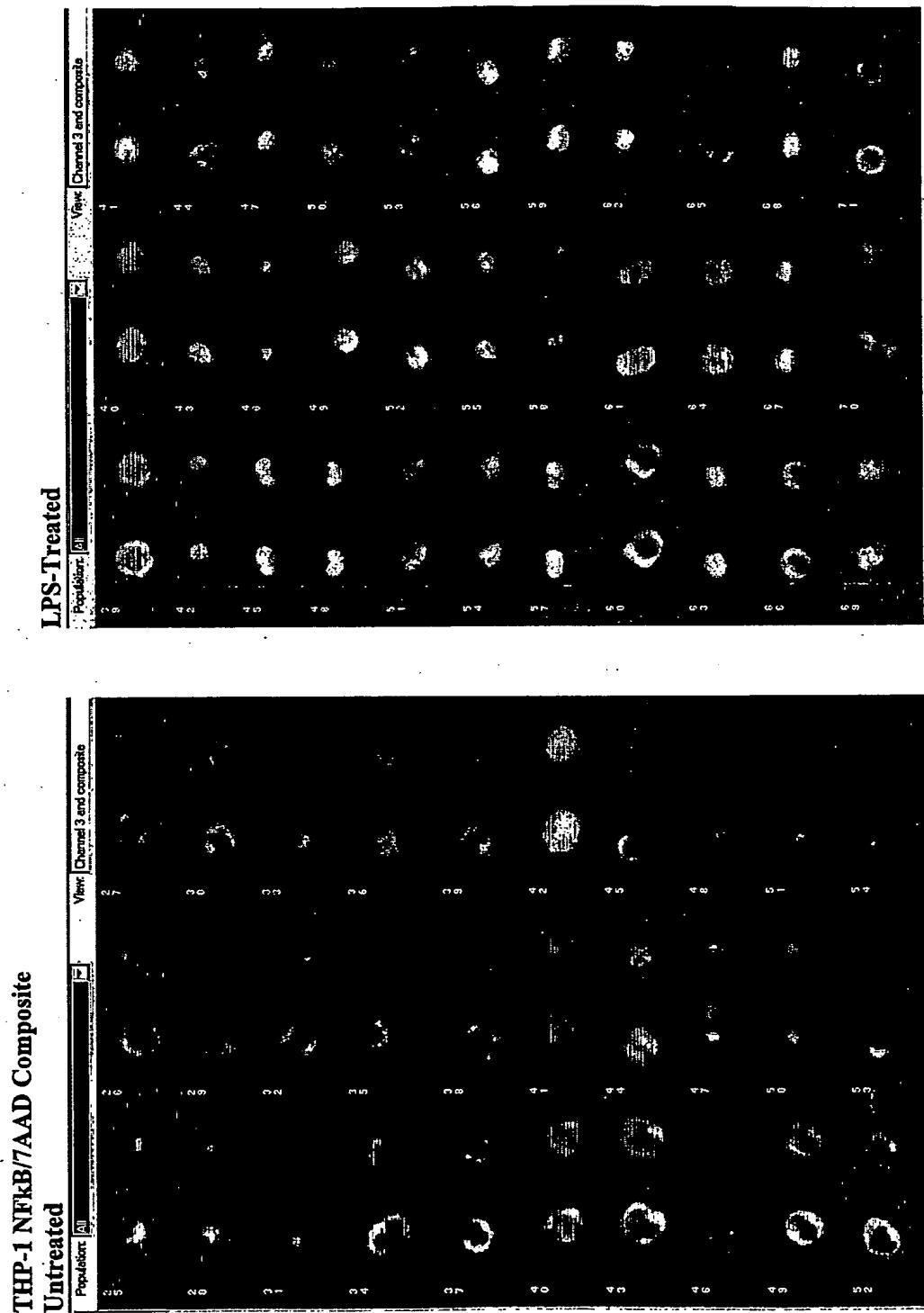
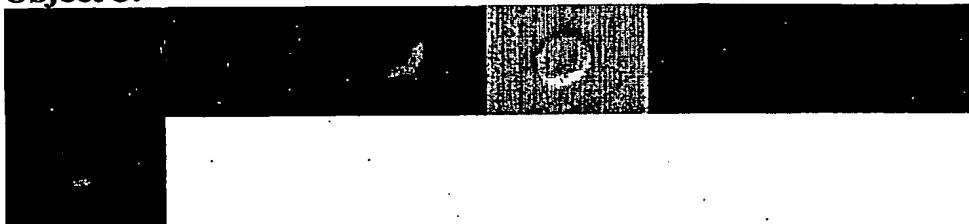
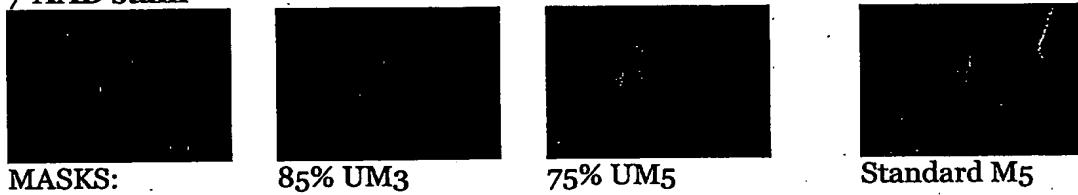
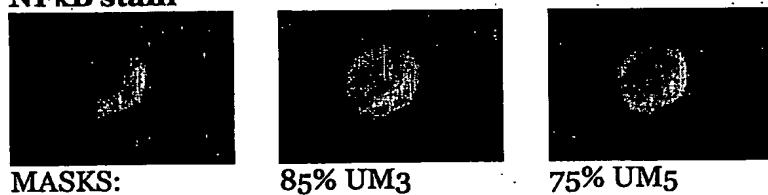


Fig. 7

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Object 8:**7-AAD stain****NF κ B stain**

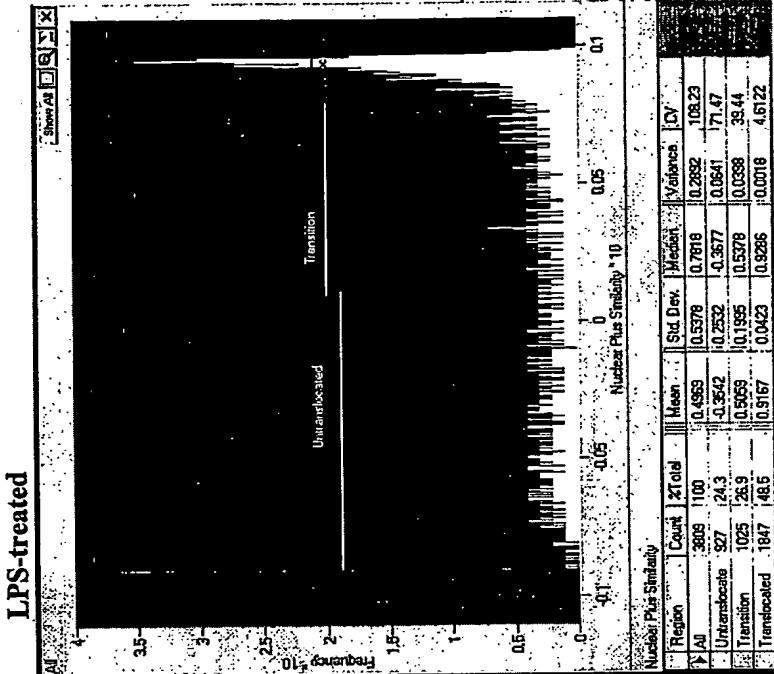
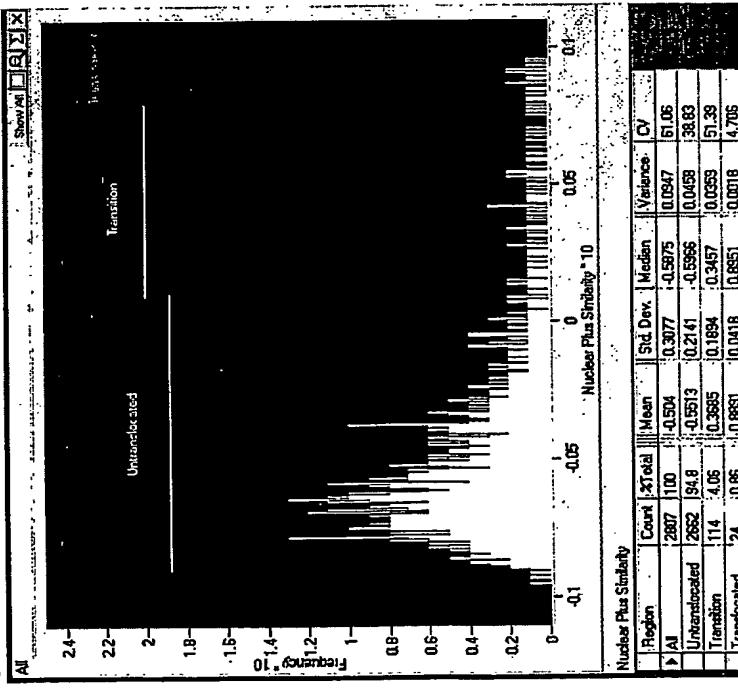
Feature	Value	Definition
3 Peak Intensity	571	Peak Intensity M3 2
Nuclear Plus Similarity	-0.155	Similarity Normalized UM5 2 4
Similarity 85 Cytoplasm	0.5003	Similarity Normalized UM3 2 4

Fig. 8

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COMPARTMENTAL CORRELATION FEATURE:

Untreated



Median Compartmental Correlation Feature:

Untranslocated = -0.5966 +/- 0.2141

Difference of 1.5252

Translocated = 0.9286 +/- 0.0423

Fig. 9

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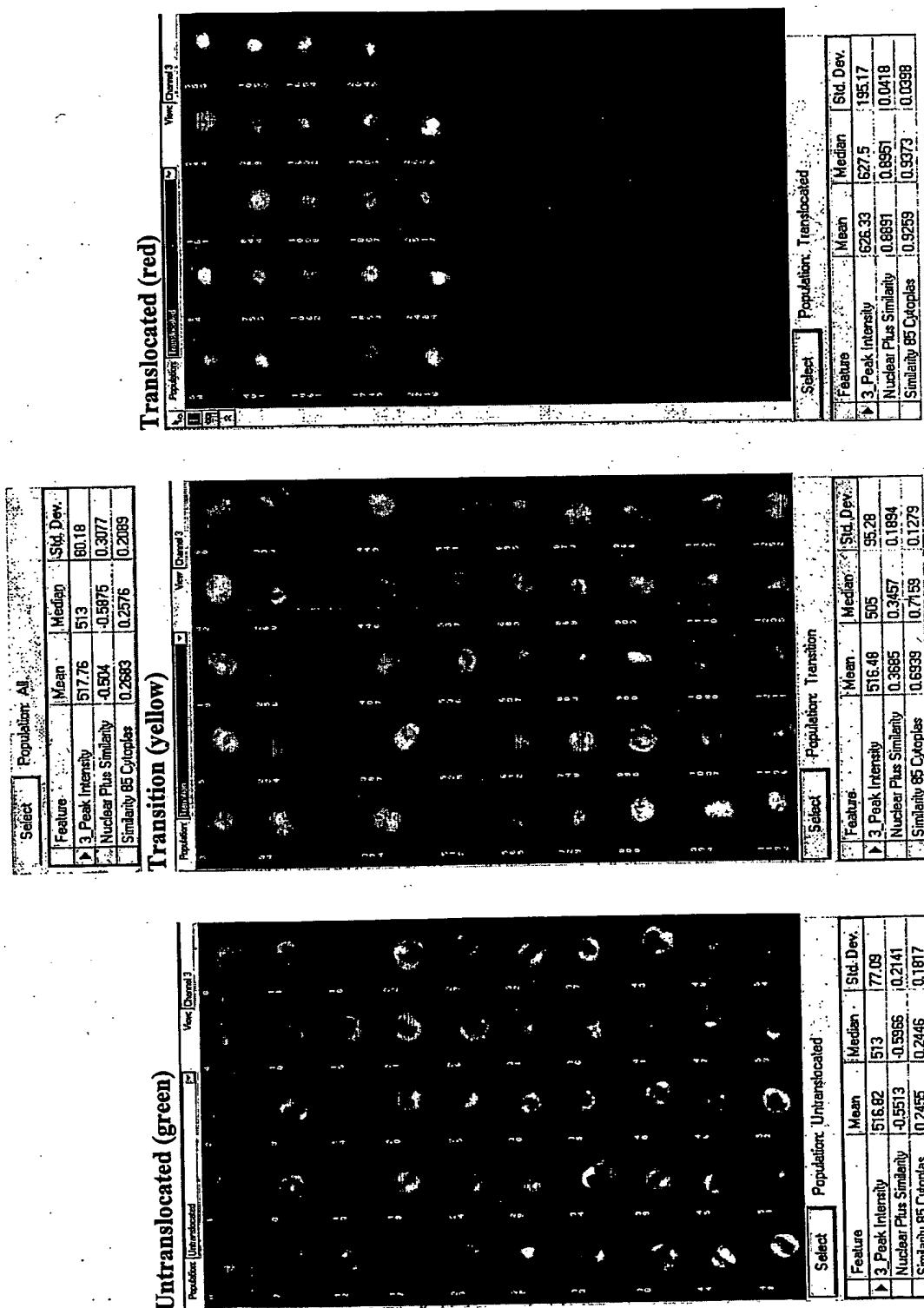


Fig. 10A

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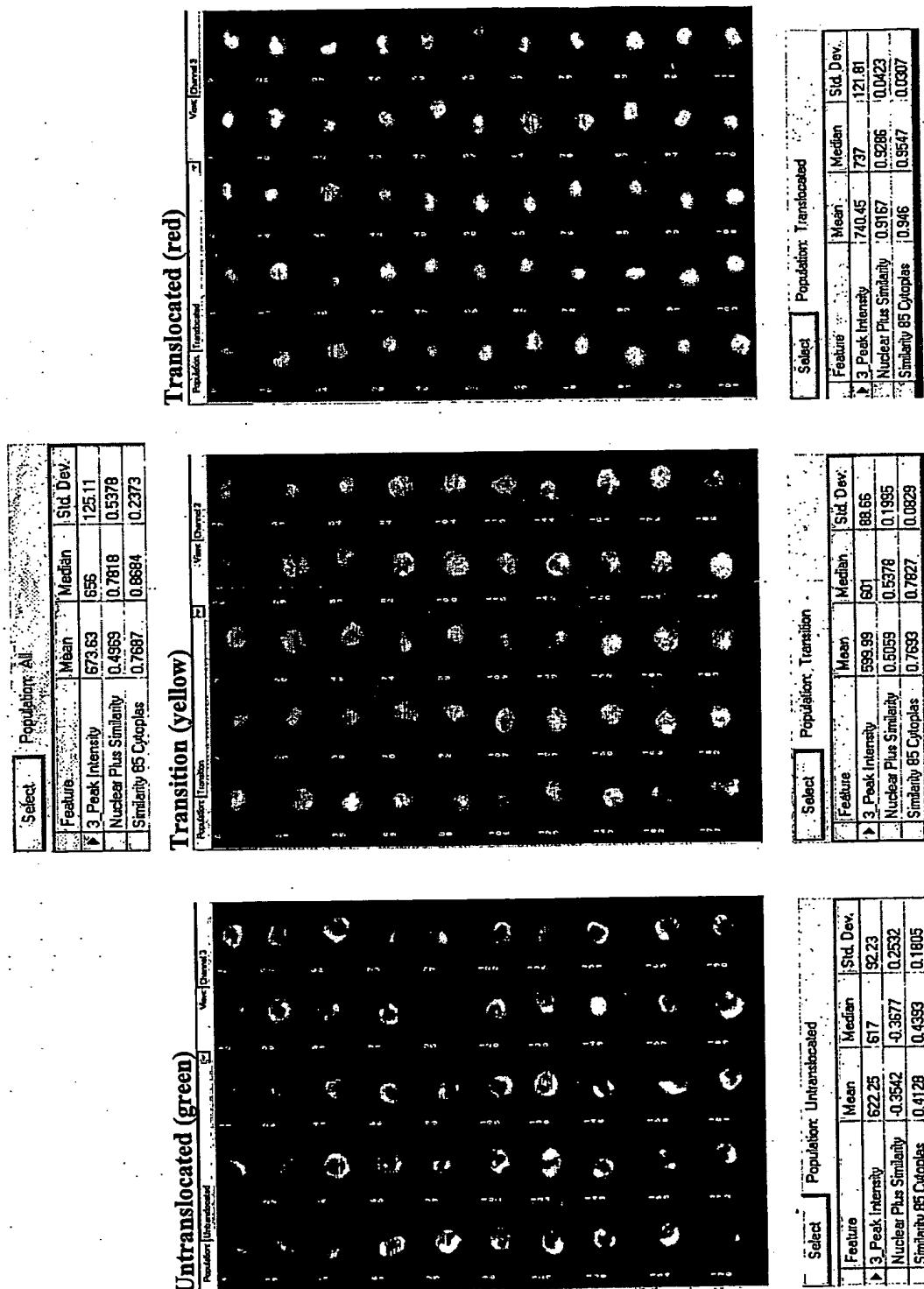


Fig. 10B

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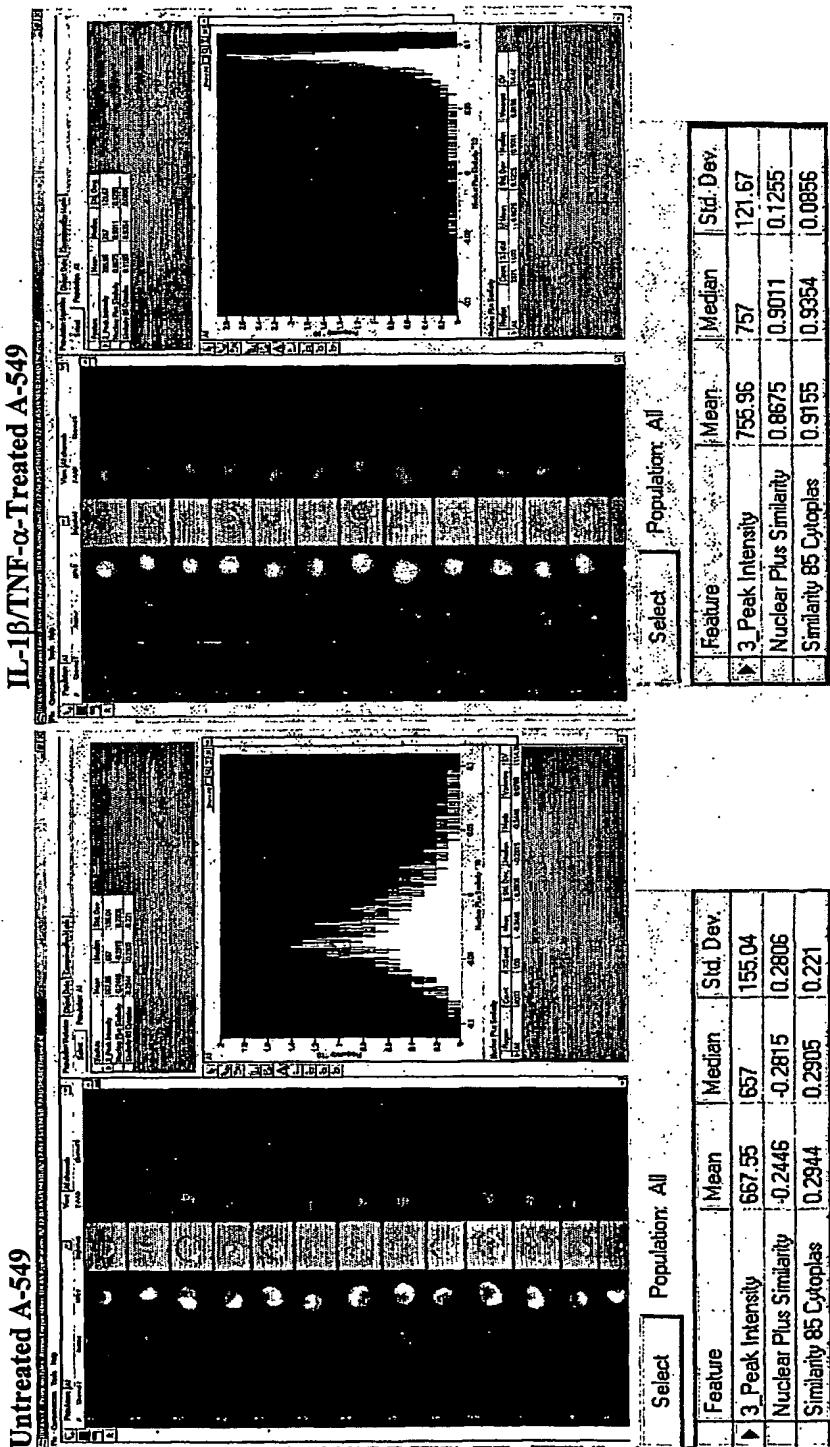


Fig. 11

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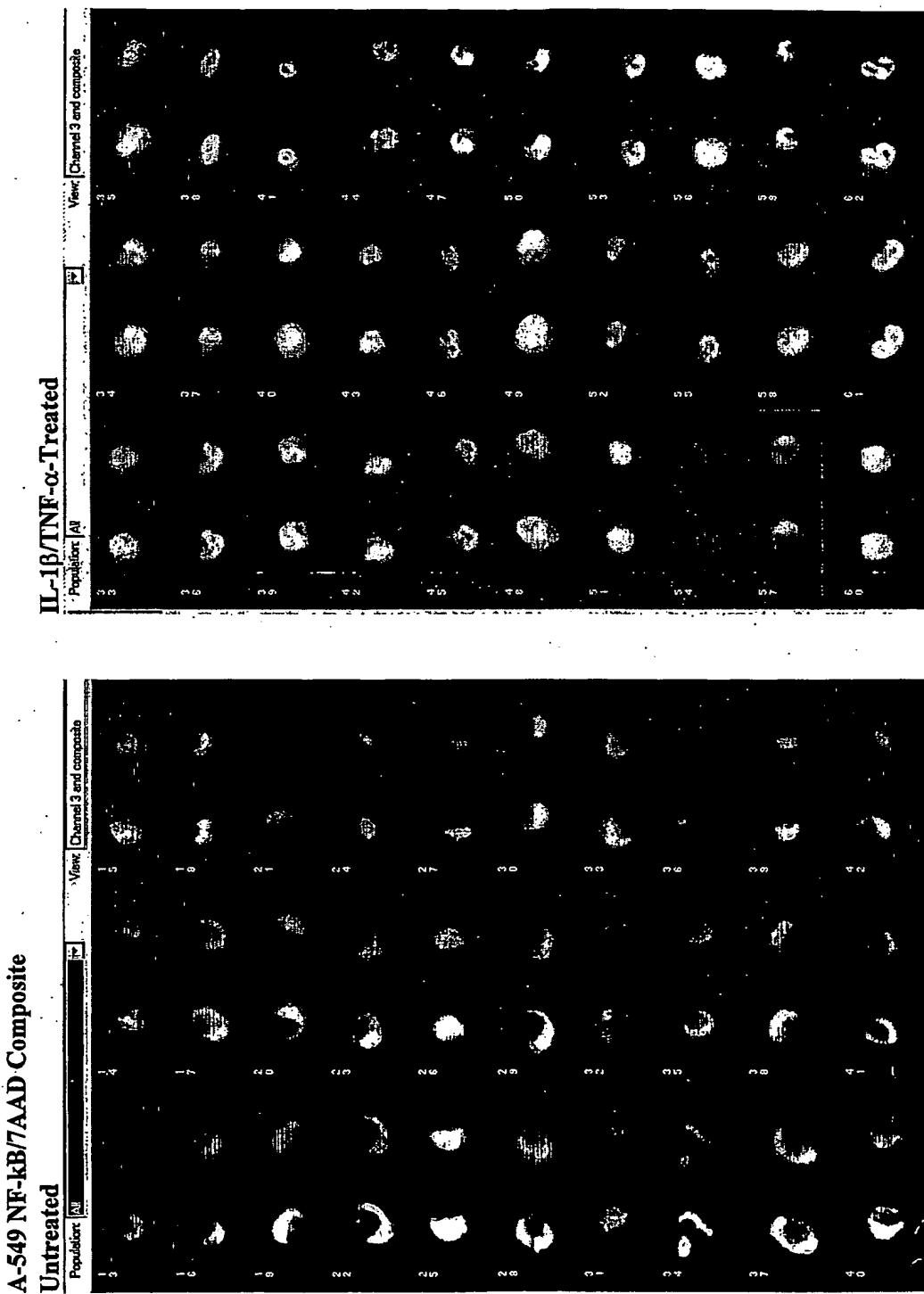
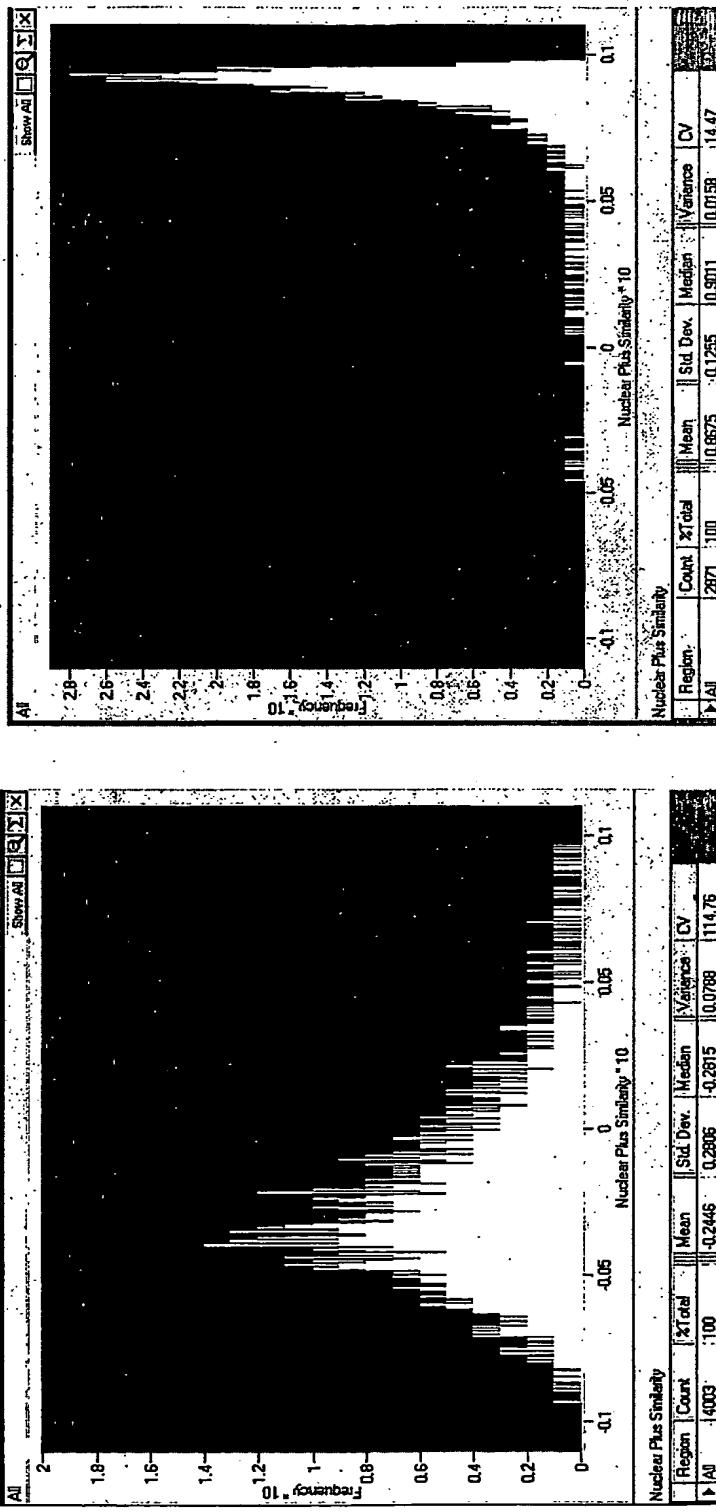


Fig. 12

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Compartmental Correlation Feature: A-549 CellsUntreatedIL-1 β /TNF- α -Treated

Median Compartmental Correlation Feature:
 -2815 ± 0.2806

Difference of 1.1826

Fig. 13